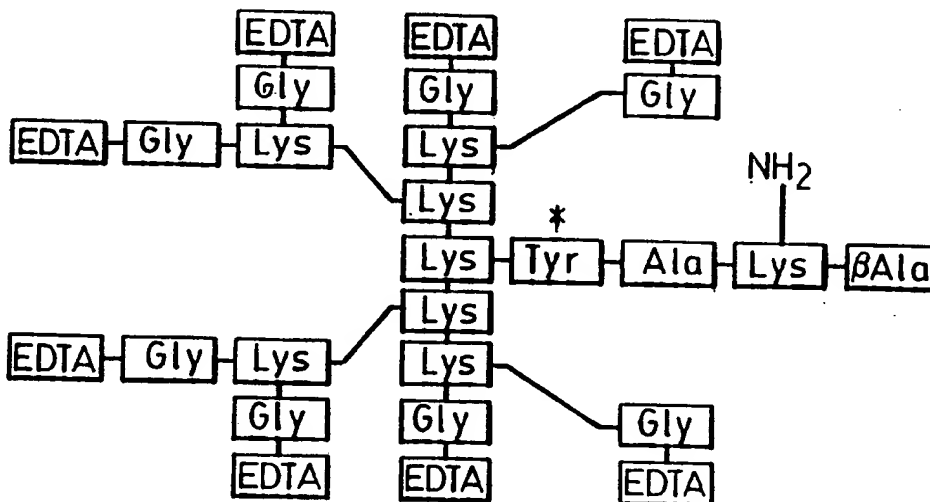




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/CA93/00207 (22) International Filing Date: 7 May 1993 (07.05.93) (30) Priority data: 07/880,691 8 May 1992 (08.05.92) US (71) Applicant: THE ONTARIO CANCER INSTITUTE [CA/CA]; 500 Sherbourne Street, Toronto, Ontario M4X 1K9 (CA). (72) Inventor: GARIEPY, Jean ; 43 Chester Avenue, Toronto, Ontario M4K 2Z8 (CA). (74) Agent: LAKE, James, R.; Ridout & Maybee, 2300 Richmond-Adelaide Centre, 101 Richmond Street West, Toronto, Ontario M5H 2J7 (CA).		(81) Designated States: JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>With amended claims.</i>

(54) Title: METAL CHELATING PEPTIDE**(57) Abstract**

The metal chelating peptide (MCP) has a C-terminus which may be structured to provide a variety of means for unidirectional coupling to a targeting agent such as an antibody. The MCP has a branched portion at its other end which contains a plurality of metal chelating sites. The number of metal chelating sites may be quite large (in excess of 16). The MCP of the invention affords the ability to deliver a concentrated radionuclide mass to a target cell by coupling the MCP to a targeting agent.

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METAL CHELATING PEPTIDE

The invention is a metal chelating peptide (MCP) which can be unidirectionally and selectively coupled to a targeting molecule such as an antibody. The peptide of the invention has a plurality of metal binding sites attached to a branched portion thereof.

Metal ions including radionuclides may be useful in diagnosis and treatment of disease. For example, α or β emitting radionuclides are useful for treatment of some cancers, and diagnostic tumor or thrombus imaging may be accomplished using paramagnetic metals in conjunction with magnetic resonance imaging or by using γ -ray emitting isotopes for visualization with γ -ray cameras. Lanthanide metal fluorescence is also useful in diagnostic testing, and the ability to create an electron dense targeting agent for electron microscopy has applications in basic research.

Delivering metal ions to a desired site, either in vivo or in vitro, and maintaining a sufficient metal ion concentration at the desired site for a sufficient time to accomplish the diagnostic or therapeutic objective, has been a goal of researchers for many years. The invention addresses this need by providing a metal chelating peptide that may take any of several structures depending on the intended application. The peptide has a reactive group at one end to unidirectionally, i.e. irreversibly, couple the peptide to the targeting molecule without affecting the inherent bioselectivity of the targeting molecule. The peptide is branched to provide a plurality of sites for attachment of metal chelating groups such as ethylenediamine tetraacetic acid (EDTA), and can be constructed using d-amino acids in order to resist degradation. Importantly, the peptides of the invention are readily synthesized using standard

techniques and may be customized in accordance with the needs of a particular application.

Typically, the metal chelating peptides of the invention will be coupled to antibodies, but may also be coupled to drugs, oligonucleotides, or peptides or other targeting molecules. In constructing antibody conjugates, the peptides may be coupled to the F_c domain of the antibody, thereby not affecting the immunoreactivity of the antibody with antigen.

Accordingly, the invention provides a metal chelating peptide which can be coupled with a targeting molecule. The peptide has a reactive group at one end to allow unidirectional and selective coupling to the target molecule, and is branched at the other end to provide a plurality of metal binding sites. A preferred reactive group for coupling the peptide is provided by a lysine residue having a free ϵ - NH_2 . Likewise, the branching of the peptide is preferably provided by a polylysine construct having free α and ϵ NH_2 groups to which metal chelating groups can be attached.

Brief Description of the Drawings

Figure 1 is a schematic representation of a general structure for an MCP of the invention.

Figure 2 is a schematic representation of a particular example of an MCP of the invention.

Figure 3 is a schematic representation of one possible pathway for coupling an MCP to an antibody.

Figures 4A-F show six possible derivatives of an MCP for use in various coupling procedures.

While the invention has broad application, it will

be described with reference to a preferred embodiment used for conjugation with antibodies such as monoclonal antibodies. Indeed, the driving force for the creation of metal chelating peptides arose from the desire to produce new generations of radioimmunoconjugates of monoclonal antibodies specific for tumor associated antigens.

With the advent of hybridoma biotechnology in the mid-1970's, there has been a worldwide research effort to develop monoclonal antibodies to tumor associated antigens. In most cases, these antigens either represent altered forms of antigens present on normal cells or are the same antigens but are over expressed on cancer cells. The therapeutic and diagnostic potential of monoclonal antibodies relates to their ability to bind tumor associated antigens. Antibodies themselves may have anti-tumor properties by virtue of immunological mechanisms such as antibody dependent cell mediated cytotoxicity, complement activation, and anti-idiotypic activity. However, rapid antigenic modulation and the lack of measurable clinical benefits from the use of antibody alone as a therapeutic agent have caused researchers to turn to an examination of immunoconjugates.

To date, three major families of immunoconjugates have been developed: drug-antibody, toxin-antibody and radionuclide-antibody conjugates. Of these, only the radioimmunoconjugates do not require endocytosis or binding to all tumor cells to deliver their toxic action. Additionally, radioimmunoconjugates are useful diagnostic tools, for example, in locating tumors.

Radioimmunoconjugates have been prepared for both therapeutic and diagnostic purposes; however, the success of such conjugates has been minimal owing to a number of factors which the present invention addresses. One problem arises from the use of monoclonal antibodies

which recognize poorly defined epitopes often located on the surface of both normal and malignant cells. Thus, other targeting agents may prove more suitable for conjugate formation. For example, peptide hormones, growth factors and lymphokines bind to defined cell surface receptors and may prove to be superior candidates to antibodies for conjugate construction in many instances.

Another problem associated with the use of radioimmunoconjugates is the need for the targeting agent to retain its binding capacity to a tumor cell marker after having been modified to carry an effective therapeutic or diagnostic probe. Past experience has shown that antibodies have often been poorly labelled with radioisotopes, thereby providing radioconjugates which are too weakly radioactive to be useful, or in which the metal is not adequately complexed leading to release of radioactive elements in vivo, or which are denatured during the conjugation process so that binding to the target antigen is impaired.

The metal chelating peptides of the invention are readily synthesized using existing or modified procedures of classical solid phase peptide synthesis. In addition to being rapid and simple, solid phase peptide synthesis permits the removal of unreacted reagents and by-products at each step of the synthesis. Also, each synthetic step may be repeated until satisfactory yields are obtained.

The peptides of the invention are branched to provide clusters of metal binding sites. These peptides may be customized for various diagnostic and therapeutic applications in association with a wide range of targeting agents.

As shown in Fig. 1, the first step in the

construction of a metal chelating peptide of the invention is to couple at least one amino acid, and preferably for most purposes several amino acids, to a solid support for the purpose of providing a molecular arm X1 which separates the metal chelating portion of the peptide from the targeting agent coupling portion of the peptide. This C-terminal end of the peptide X1 has a reactive moiety permitting the unidirectional coupling of the peptide to a targeting agent. Thus, the molecular arm X1 may have a free carboxyl or amide end and may have a hydrazide, azide, activated ester, thiol, aldehyde, amino, maleimido, isothiocyanate, haloacetate, photo-reactive or other reactive group capable of effecting the desired unidirectional coupling to the targeting agent.

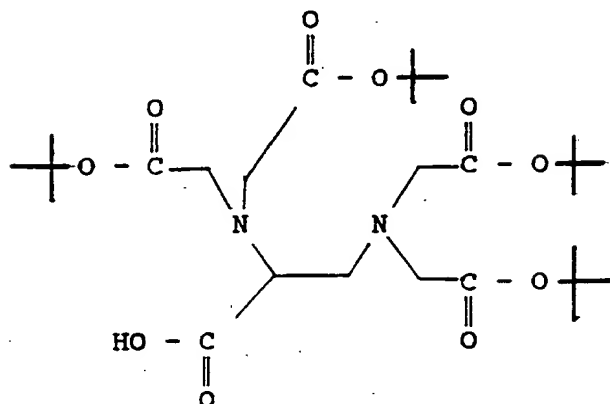
The second part of the peptide of the invention is a branched structure formed from diamino carboxylic acids such as lysine, ornithine, or 1,3-diaminopropionic acid. Referring to Fig. 1, the branched structure is connected to the C-terminus X1 via the spacer X2 which may be omitted or may be up to a few amino acids in length depending on the spacer requirements for separating the branched portion of the peptide from the targeting agent coupling site at the C-terminus X1.

In Fig. 1, X3 represents a branch forming amino acid, preferably a diamino acid such as lysine, ornithine or 1,3-diaminopropionic acid. The group X4 in Fig. 1 provides the capability for the attachment of a plurality of chelation sites X5. The preferred branch forming amino acid is L-lysine; however, the use of D-lysine and D-amino acids for construction of the peptide will reduce the rate of degradation of the peptide in vivo, and the use of shorter diaminocarboxylic acids for branching produce a more compact structure.

When the desired number of amino groups have been

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assembled onto the peptide, a metal chelator X5 is attached to each amino group. A preferred metal chelator is ethylenediamine tetraacetic acid (EDTA), which may be coupled to the amino end of an amino acid by using the derivative I, the simple



I

synthesis of which is reported in Arya, R. and Gariépy, J. (1991) *Bioconjugate Chemistry* 2, 323-326. The advantage of using an EDTA derivative of the type I is that the full EDTA structure is available for metal chelation as a hexadentate ligand. The skilled person will appreciate that a similar chelation strategy can be employed using other chelators such as diethylene-triamine pentaacetic acid.

The following is a typical synthetic scheme for preparation of a peptide of the invention. The peptide, termed MCP-8-EDTA-NH₂, is shown in Fig. 2.

Preparation of Metal Chelating Peptide, MCP-8-EDTA-NH₂

The peptide shown in Fig. 2 was prepared by solid phase peptide synthesis on an automated Applied Biosystems model 430 Peptide Synthesizer using t-Boc protected amino acids and PAM resin supports. Unless

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indicated, all couplings were carried out using symmetric anhydride derivatives of protected amino acids. All synthesis protocols employed were those established by the manufacturer (Applied Biosystems, Foster City, CA). Each coupling step was monitored by the quantitative determination of free amino groups present on the resin (quantitative ninhydrin test). Typically, the efficiency of each coupling step was close to 99%. As illustrated in Figure 2, the first residue coupled to the PAM resin was β -alanine (β -aminopropionic acid) and the substitution on the resin support was 0.1 mmole/gram resin. The initial low substitution value on the resin insures that crowding of the resin with peptide chains will not occur as a result of three branching steps (i.e. maximal substitution of $2^3 \times 0.1 \text{ mmole} = 0.8 \text{ mmole/gram resin}$). The β -alanine serves as an internal standard. The second residue is a lysine (N α -Boc, N ϵ -Fmoc-Lysine) having its ϵ -amino group protected with a 9-fluoromethoxycarbonyl amino group (Fmoc). The base-sensitive Fmoc group provides an option to introduce a reactive site at this position on the resin prior to chain cleavage from the resin. A more classical lysine derivative with an acid labile side chain protecting group (cleavable with HF; 2 ClZ derivative for example) has also been used. A protected cysteine residue (for example Cys (Acm)) could have been introduced at this position if a thiol group would have been preferred later for conjugation purposes (for labelling mAb fragments for example). The third and fourth residues coupled were alanine and tyrosine respectively. These residues act as molecular spacers between the peptide branches and the C-terminus reactive group. They also permit one to monitor the quality of the synthesis when samples taken at each step during the construction of the polymer are analyzed by amino acid analysis. In addition, the aromatic side chain of tyrosine allows for the determination spectroscopically the concentration of the peptide stock

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solutions and can be radioiodinated providing an approach to determine the number of peptides coupled to antibodies. The first through fourth residues here correspond to the -X2-X1 chain of Fig. 1. The fifth residue was $N\alpha,N\epsilon$ -Boc-lysine(Boc), an amino acid having its amino groups at the $C\alpha$ and $C\epsilon$ positions protected with acid labile Boc protecting groups. After deprotecting these sites with TFA, branching is initiated by coupling two equivalents of $N\alpha,N\epsilon$ -Boc-lysine(Boc) to the two available amino positions. After another round of acid deprotection, the branching step was repeated with this time four amino sites available for coupling. $N\alpha,N\epsilon$ -Boc-lysine(Boc) and glycine(Boc) were successively coupled again. Upon removal of the Boc groups, eight new amino sites were exposed. The t-butyl protected EDTA carboxy derivatives I were then coupled to these amino sites (2 molar excess over the number of moles of free amino groups) in the presence of an equivalent molar amount each of dicyclohexylcarbodiimide and N-hydroxybenzotriazole in 50% DMF/DCM. The reaction was performed at room temperature for 2 hours. This coupling step was repeated and the completion of this reaction as monitored by quantitative ninhydrin proved to be greater than 99%. Any remaining unreacted amino groups were acylated with acetyl anhydride in the presence of diisopropylethylamine. The t-butyl groups were cleaved in 50% TFA/DCM for 2 hours and the protecting groups removed in the presence of trifluoroacetic acid (TFA). The peptide-resin was then treated with 20% piperidine/DMF for 20 minutes to deblock the Fmoc group present on the ϵ -amino group of the C-terminus lysine. Finally, the MCP-8-EDTA-NH₂ (Fig. 2) was cleaved from the support with 10% anisole/anhydrous hydrogen fluoride at 0°C for 45 minutes. The resin was extracted with several ether washes to remove the anisole and the cleaved protecting groups. The branched peptide was then recovered by extracting the resin with 100% TFA and

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rapidly evaporating the acid. The peptide was then suspended in 50 mM ammonium bicarbonate and lyophilized.

The skilled person will appreciate that the number of amino groups available for the introduction of chelator sites can be increased readily to 16 or more, with the upper limit depending on the ability to resolve problems of low coupling rates associated with the crowding of the resin support and the accessibility of free amino groups. The rapidity of the synthesis strategy (two days) and its potential for automation represent major assets in developing the technology. With an expected molecular weight of 4307 daltons, MCP-8-EDTA-NH₂ is desalted initially on a Sephadex G-10 column to remove any low molecular weight impurities resulting from the cleavage step. The recovered polymer has 8 (+/- 2) EDTA groups and the proper amino acid composition. The chelating properties of MCP-8-EDTA-NH₂ has also been confirmed by the binding of radionuclides such as ^{99m}Tc.

Coupling of MCP-8-EDTA to the carbohydrate domain of monoclonal antibodies

Aldehyde groups can be generated on monoclonal antibodies by the periodate oxidation of carbohydrate moieties located in the Fc domain. These sites have been recently exploited to selectively label antibodies and glycoproteins with hydrazide derivatives resulting in a minimal loss of activity.

Introducing a maleimide functionality on MCP-8-EDTA-NH₂

A number of strategies can be devised to couple peptides of the invention to antibodies. This flexibility is one of the powerful features of the invention. Fig. 4 illustrates 6 examples of how simple

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modifications of the peptide can lead to alternate approaches for coupling it to a targeting agent. In the selected example outline in Fig. 3, MCP-8-EDTA-NH₂ is first reacted with the bifunctional crosslinking agent, m-maleimido-benzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBS; Fig. 3, pathway a). Typically 3 mg of sulfo-MBS dissolved in PBS is mixed with 1 mg of the peptide dissolved in PBS. The reaction is left to process at room temperature for 1 hour with occasional stirring. The polymer-MBS is desalted from unreacted sulfo-MBS by gel filtration using Sephadex G-25. Using the reactivity of the resultant conjugate in a quantitative DTNB/β-mercaptoethanol assay, it has been confirmed that one mole of MBS is coupled to one mole of the peptide under these conditions. This compound, abbreviated as MCP-8-EDTA-NH-MB, is stable for days in aqueous buffers (PBS) at 4°C.

Construction of MCP-antibody conjugates

The maleimide derivative of the peptide (MCP-8-EDTA-NH-MB) selectively and rapidly reacts with any free thiols present on the surface of targeting agents. As an example, thiol groups can be created on an antibody by partially reducing with DTT one or more of the disulfide bridges present at the hinge region of IgG molecules. Alternatively, the Fc domain of antibodies can be targeted by oxidizing the carbohydrate moieties located on each heavy chain to create free aldehyde groups (Fig. 3). Indeed, in the presence of sodium periodate, aldehyde groups are generated which can then be readily reacted with a thiol-containing hydrazide compound such as 2-acetamido-4-mercaptobutyric acid hydrazine (Fig. 3, pathway b). The resulting thiol-containing antibody preparation can then be reacted with MCP-8-EDTA-NH-MB (50-fold excess in relation to the number of moles of antibody) overnight at room temperature in an aqueous

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buffered solution adjusted to a pH between 4 and 8 (Fig. 3, pathway c). The complex is then desalted on Protein PakI 125 (Waters; Millipore) HPLC gel filtration column from the unreacted MCP-8-EDTA-NH-MB.

Construction of MCP-antibody fragment conjugates

Amino groups on $F(ab')_2$ fragments can be linked through a bifunctional crosslinker to the MCP-8-EDTA carrying a cysteine moiety (Fig. 4A as an example), while the single reactive thiol group of $F(ab')$ fragments will be coupled to the peptide carrying a lysine group (Fig. 4B as an example). A variety of commercially available heterobifunctional crosslinkers containing a maleimido group at one end (reacts with thiol group) and a succinimidyl ester group at the other (reacts with amino groups) can be used to link together MCPs and mAb fragments. In the case of $F(ab')_2$ -MCP conjugates, the antibody fragment will first be reacted with MBS and the resulting construct purified by gel filtration (Sephadex G-50). Excess MCPs (50-fold molar excess over amino groups on fragment) containing a free thiol group is then added to the desalted fragment-MBS conjugate and the reaction allowed to proceed for 2 hours at room temperature (with occasional stirring). In all cases [$F(ab')$ and $F(ab')_2$ conjugates], the final conjugates can be purified from the excess polymer by gel filtration (Sephadex G-50).

Variations of the basic concept lead to the design of a multitude of MCPs

Fig. 4 illustrates six approaches to link MCPs to targeting agents. In Fig. 4, only a few variations of the basic polymer are shown that would permit an MCP to be coupled unidirectionally to amino, thiol, or carbohydrate groups present on a targeting agent. Non-

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specific labelling strategies such as the use of photoreactive groups (aryl azides or benzoyl benzoate derivatives, for example, Fig. 4F) are also illustrated.

From the foregoing description and examples, the skilled person will appreciate that the invention is of broader scope than the specific peptides illustrated. A significant strength of the invention is its versatility and wide applicability to a variety of metal chelating applications.

CLAIMS:

1. A metal chelating peptide, comprising:

a carboxy-terminal portion having 3-6 amino acid residues, wherein only the amino acid residue adjacent the C-terminal residue has a reactive group for unidirectionally and selectively coupling the peptide to a targeting molecule; and

a branched N-terminal portion having 4-16 amino groups to which are coupled metal chelating polydentate ligands.

2. A peptide as claimed in claim 1, wherein the reactive group is hydrazide, azide, activated ester, thiol, aldehyde, amino, maleimido, isothiocyanate, haloacetate or a photoreactive group.
3. A peptide as claimed in claim 1, wherein the branched portion is formed by coupling a plurality of diamino carboxylic acids.
4. A peptide as claimed in claim 3, wherein the diamino carboxylic acids are spaced from one another by one to three monoamino carboxylic acids.
5. A peptide as claimed in claim 3, wherein the diamino carboxylic acids are lysine, ornithine or 1,3-diaminopropionic acid.
6. A peptide as claimed in claim 1, wherein each metal chelating ligand comprises an ethylenediamine tetraacetic acid hexadentate ligand after coupling to the peptide.
7. A peptide as claimed in claim 1, wherein the amino acid residue adjacent the C-terminal residue is a

diaminocarboxylic acid or cysteine.

8. A peptide as claimed in claim 1, wherein the amino acid residue adjacent the C-terminal residue is D or L lysine.

9. A peptide as claimed in claim 1, wherein the C-terminal residue is β -alanine.

10. A peptide as claimed in claim 1, wherein the carboxy-terminal portion is TyrAlaLys β Ala.

AMENDED CLAIMS

[received by the International Bureau on 7 September 1993 (07.09.93);
original claim 1 amended; remaining claims
unchanged (2 pages)]

1. A metal chelating peptide, comprising:

a linear carboxy-terminal portion having 3-6 amino acid residues, wherein said portion has a reactive group for unidirectionally and selectively coupling the peptide to a bioselective targeting molecule selected from the group: antibodies, antigens, oligonucleoties and drugs; and

a branched N-terminal portion having 2-16 amino groups to which are coupled metal chelating polydentate ligands.

2. A peptide as claimed in claim 1, wherein the reactive group is hydrazide, azide, activated ester, thiol, aldehyde, amino, maleimido, isothiocyanate, haloacetate or a photoreactive group.

3. A peptide as claimed in claim 1, wherein the branched portion is formed by coupling a plurality of diamino carboxylic acids.

4. A peptide as claimed in claim 3, wherein the diamino carboxylic acids are spaced from one another by one to three monoamino carboxylic acids.

5. A peptide as claimed in claim 3, wherein the diamino carboxylic acids are lysine, ornithine or 1,3-diaminopropionic acid.

6. A peptide as claimed in claim 1, wherein each metal chelating ligand comprises an ethylenediamine tetraacetic acid hexadentate ligand after coupling to the peptide.

7. A peptide as claimed in claim 1, wherein the amino

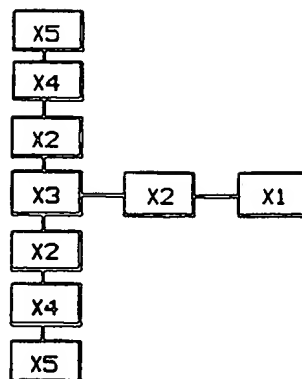
acid residue adjacent the C-terminal residue is a diaminocarboxylic acid or cysteine.

8. A peptide as claimed in claim 1, wherein the amino acid residue adjacent the C-terminal residue is D or L lysine.

9. A peptide as claimed in claim 1, wherein the C-terminal residue is β -alanine.

10. A peptide as claimed in claim 1, wherein the carboxy-terminal portion is TyrAlaLys β Ala.

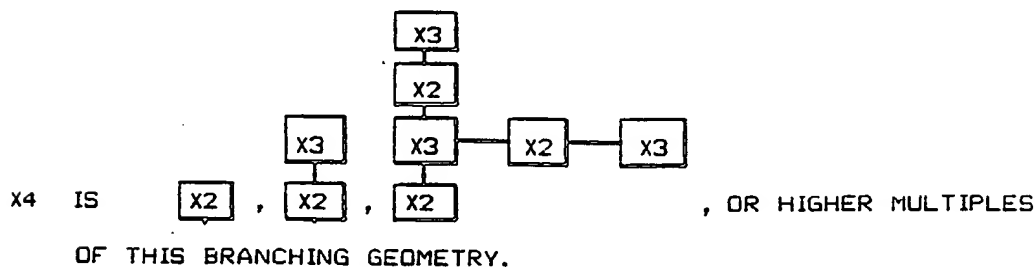
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WHERE X1 IS AN AMINO ACID, SHORT PEPTIDE OR OTHER MOLECULAR SPACER THAT HAS A FREE CARBOXYL OR AMIDE END AND CARRIES EITHER A HYDRAZIDE, AZIDE, ACTIVATED ESTER, THIOL, ALDEHYDE, AMINO, MALEIMIDO, ISOTHIOCYANATE, HALOACETATE, PHOTOREACTIVE GROUP OR OTHER REACTIVE MOIETY PERMITTING THE UNIDIRECTIONAL COUPLING TO A TARGETING AGENT.

X2 IS AN AMINO ACID, SHORT PEPTIDE OR OTHER MOLECULAR SPACER, OR X2 MAY REPRESENT NO ATOMS.

X3 REPRESENTS ANY DIAMINOCARBOXYLIC ACID COMPOUND SUCH AS (D OR L) LYSINE, (D OR L) ORNITHINE OR (D OR L) DIAMINOPROPIONIC ACID.



X5 IS $\left\{ \text{X2} - \text{CHELATOR} \right\}_n$ WHERE CHELATOR REPRESENTS METAL CHELATING GROUPS SUCH AS EDTA, DIAMINO DITHIOL CONTAINING MOIETIES, OR DTPA.

n REPRESENTS THE NUMBER OF FREE AMINO GROUPS GENERATED BY THE PREVIOUS ADDITION OF X4.

FIG. 1

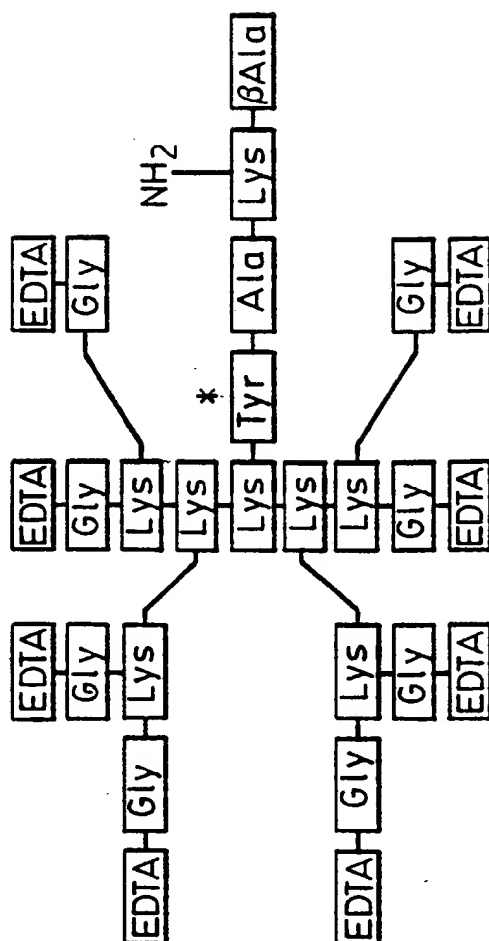
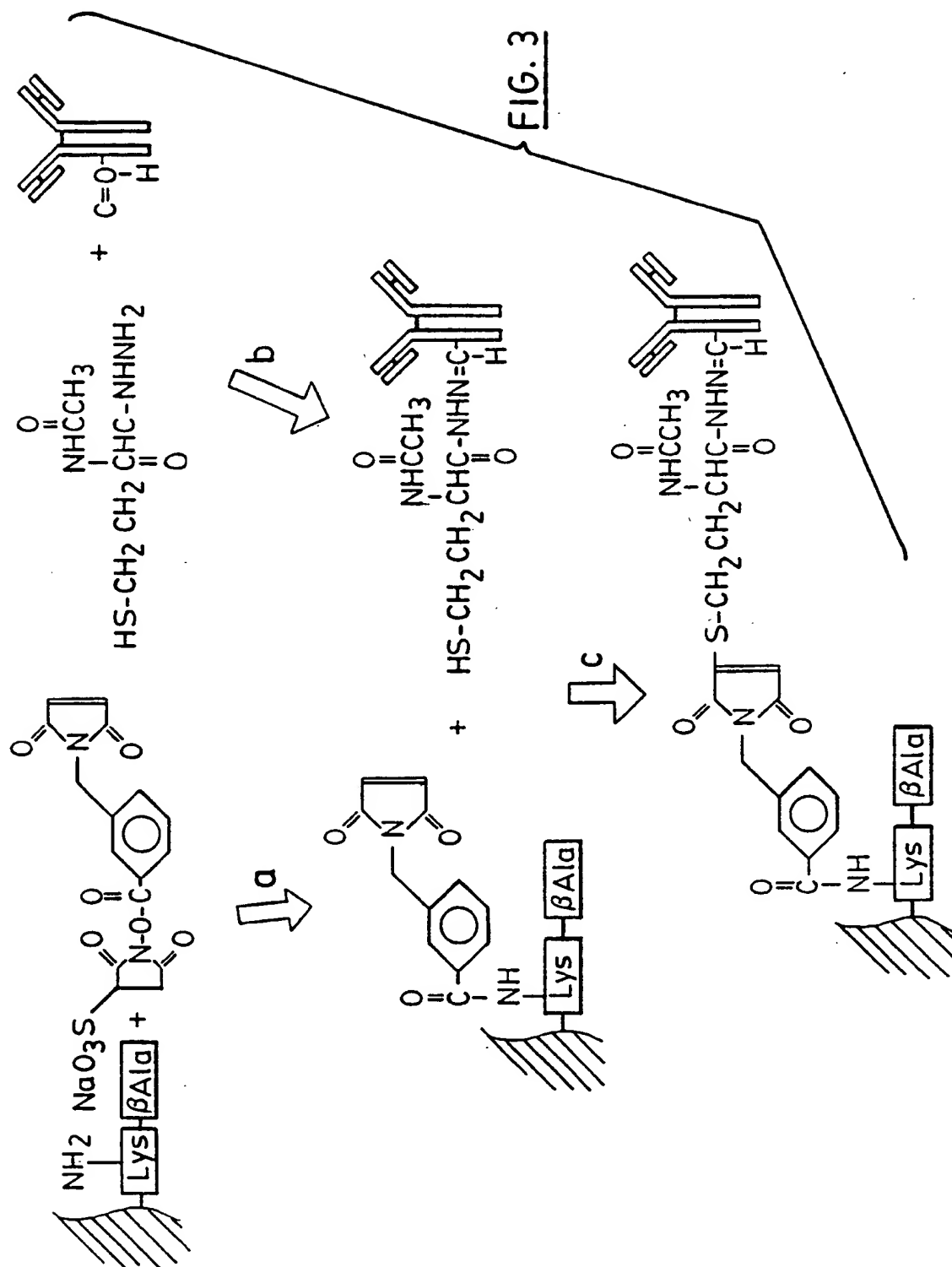
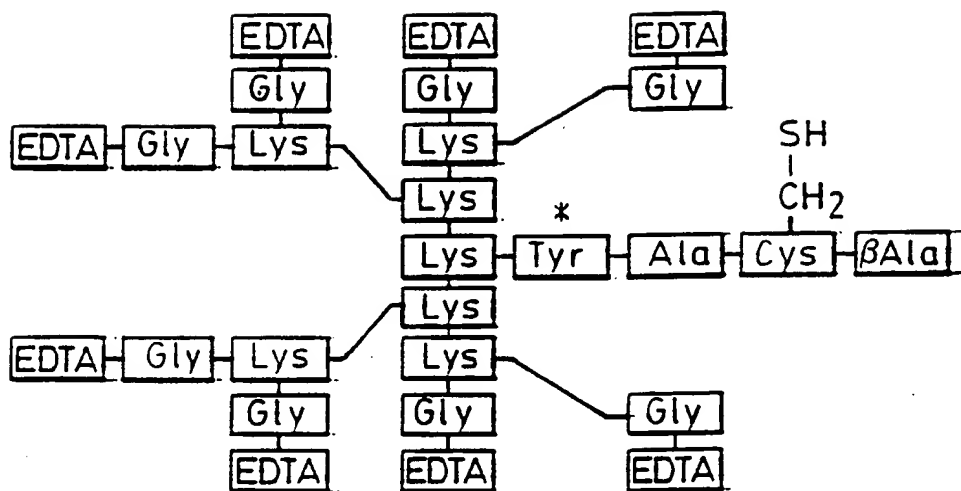
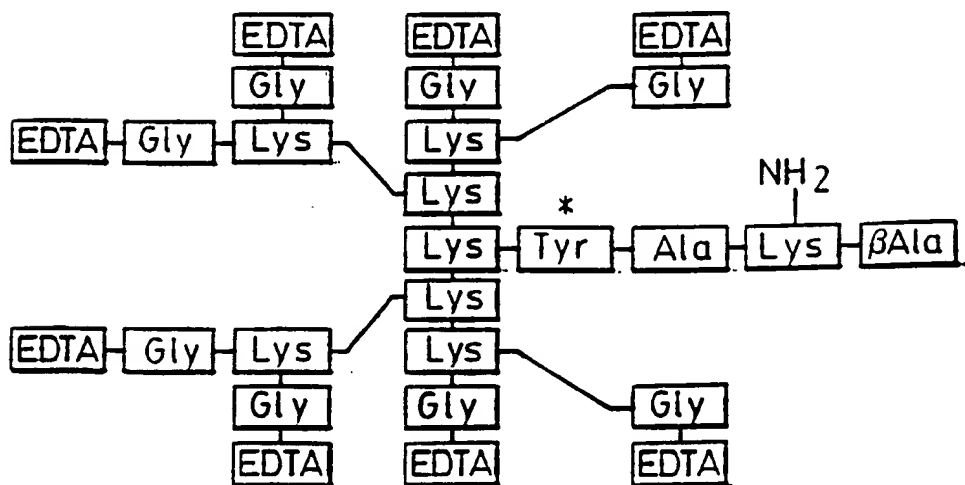
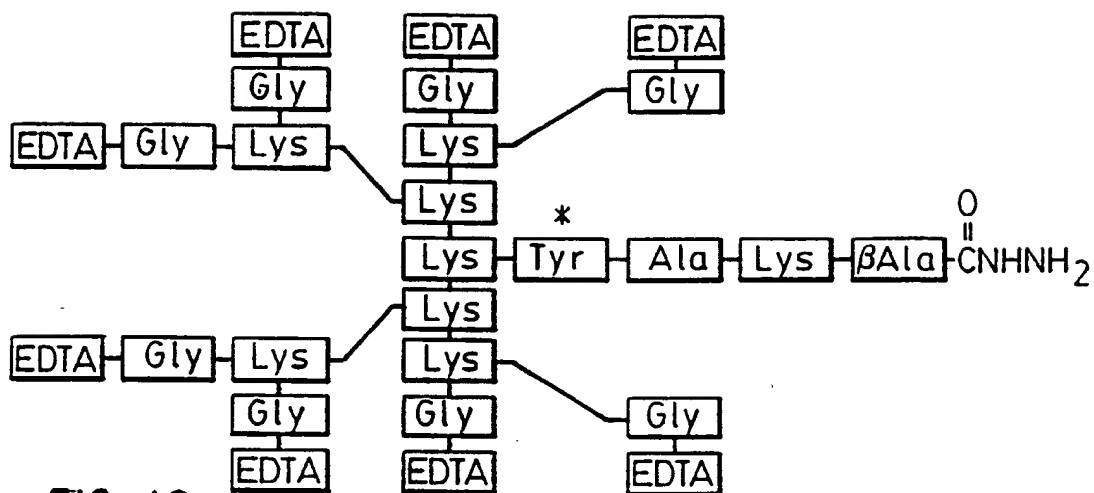
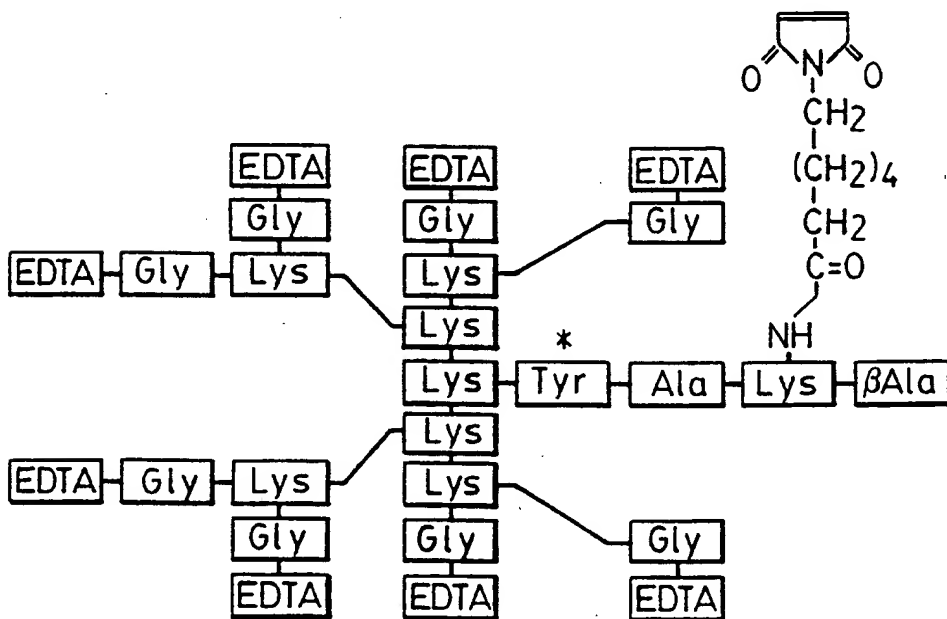


FIG. 2



FIG. 4AFIG. 4B

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FIG. 4CFIG. 4D

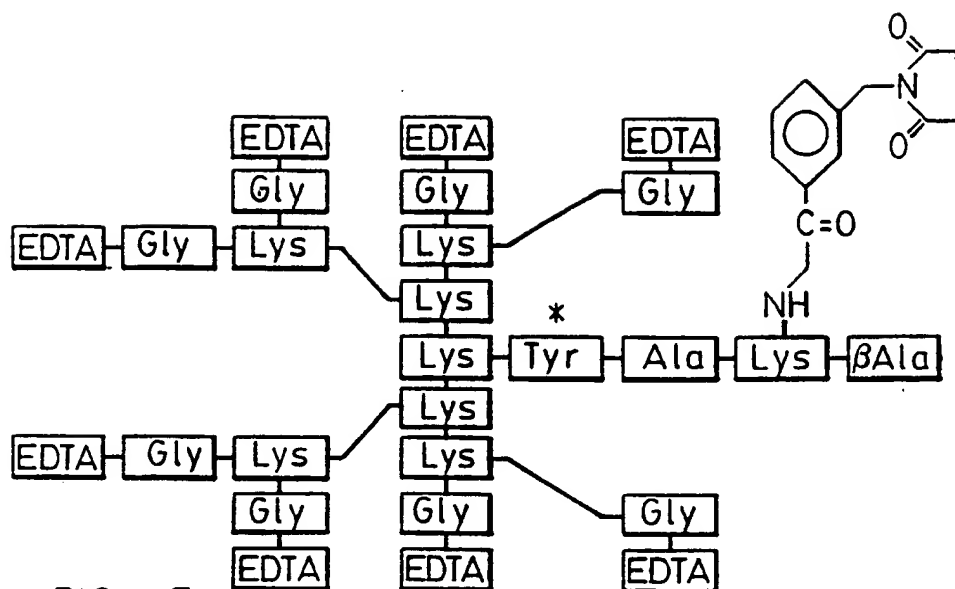


FIG. 4E

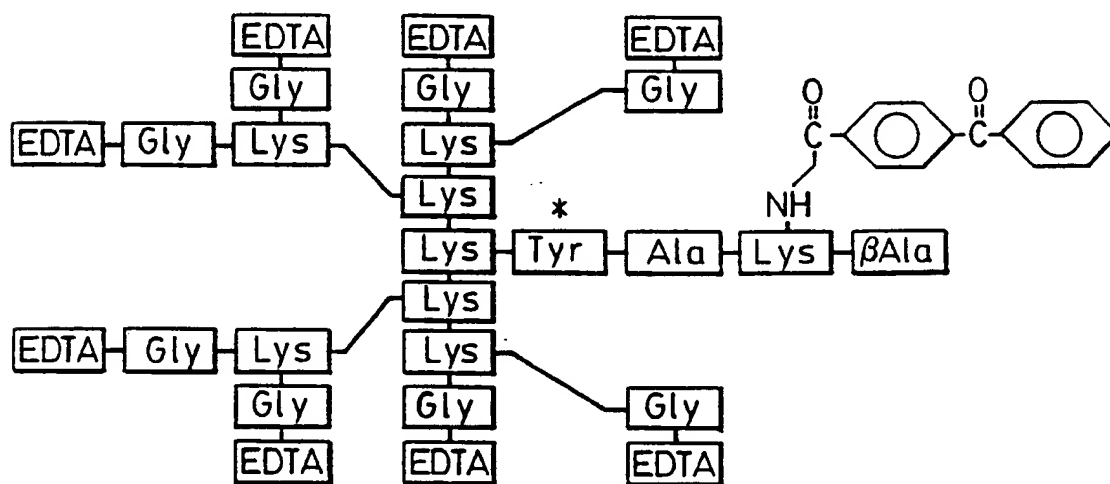


FIG. 4F

INTERNATIONAL SEARCH REPORT

PCT/CA 93/00207

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C07K7/04; G01N33/534; G01N33/68		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C07K ; G01N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	WO,A,9 205 802 (NEORX CORPORATION) 16 April 1992 * pages 1 and 2; figure 1; claim 1 * ---	1-10
A	EP,A,0 419 387 (IMMUNOTECH PARTNERS) 27 March 1991 ---	
A	WO,A,9 101 144 (SANDOZ) 7 February 1991 -----	
<p>¹⁰ Special categories of cited documents :¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
20 JULY 1993	2. 08. 93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	HERMANN R.	

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

CA 9300207
SA 74506

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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